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REDUCTION OF OCULAR COUNTER-ROLLING BY ADAPTATION TO SPACE

Mingjia Dai<sup>1</sup>, Leigh McGarvie<sup>1</sup>, Inessa Kozlovskaya<sup>2</sup>, Mischa Sirota<sup>2</sup> Theodore Raphan<sup>3</sup> and  
Bernard Cohen<sup>1</sup>

From the Departments of Neurology and Physiology and Biophysics, Mount Sinai School of  
Medicine<sup>1</sup>, New York; The Institute of Biomedical Problems, Moscow<sup>2</sup>; and the Department of  
Computer and Information Science, Brooklyn College, CUNY<sup>3</sup>

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Correspondence and Reprint Requests to:

Dr. Mingjia Dai  
Department of Neurology, Box 1135  
Mount Sinai School of Medicine  
1 East 100th Street  
New York, NY 10029 USA  
Tel: 212 241 7068; FAX 212 831 1610

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## SUMMARY

We studied the three-dimensional vestibulo-ocular reflex (VOR) of rhesus monkeys before and after the COSMOS Biosatellite 2229 Mission of 1992-1993. This included tests of ocular counter-rolling (OCR), the gain of the vestibulo-ocular reflex (VOR) and spatial orientation of velocity storage. A four-axis vestibular and oculomotor stimulator was transported to the Institute of Biomedical Problems in Moscow for the pre- and postflight ground-based testing. Twelve normal juvenile male rhesus monkeys were implanted surgically with eye coils and tested 60-90 days before spaceflight. Two monkeys (7906 and 6151), selected from the twelve as flight animals, flew from 12/29/92 to 1/10/93. Upon recovery, they were tested for 11 days postflight along with three control animals.

Compensatory ocular torsion was produced in two ways: 1) Lateral head tilts evoked Ocular Counter-Rolling (OCR) through otolith-ocular reflexes. OCR was also measured dynamically during off-vertical axis rotation (OVAR). 2) Rotation about a naso-occipital axis that was either vertical or horizontal elicited torsional nystagmus through semicircular canal-ocular reflexes (Roll VOR). OCR from the otoliths was substantially reduced (70%) for 11 days after reentry on both modes of testing. The gain of the Roll VOR was also decreased, but less than OCR. These data demonstrate that there was a long-lasting depression of torsional or roll eye movements after adaptation to microgravity in these monkeys, especially those movements produced by the otolith organs.

The gain of the horizontal or yaw VOR was close to unity when the two animals were tested 1 and 2 days after landing, respectively. The VOR gain value was similar to those registered before flight. This result confirms previous findings from the 1989 COSMOS 2044 Mission (Cohen et al. 1992). It indicates that there are no long-term changes in the passive gain of the yaw VOR on return after adaptation to microgravity.

The gain of the vertical or pitch VOR were not changed if values from the up- and downward nystagmus were pooled. However, there was a slight, but statistically significant decrease in upward VOR gain ( $p < 0.05$ ), and there was a slight but statistically significant increase in downward VOR gain ( $p < 0.05$ ). This reduced the asymmetry of up-down nystagmus that had existed prior to flight in these monkeys. The downward gain returned to the preflight level for 6151, but not for 7906 7 days after reentry.

We also examined the spatial orientation of velocity storage, using optokinetic nystagmus (OKN) and after-nystagmus (OKAN), according to techniques developed previously (Raphan and Sturm, 1991; Dai et al. 1991). Before flight, the yaw axis eigenvector angle was  $5^\circ$  with regard to the spatial vertical for monkey 7906 when the animal was tilted  $90^\circ$ . That is, the yaw axis orientation vectors of velocity storage were closely aligned to the spatial vertical before flight. One day after landing the same test yielded an orientation vector angle of  $28^\circ$  re the spatial vertical, which was a significant shift of the yaw axis eigenvector toward the body axis. By 7 days after recovery, the orientation vector angle had returned to  $7^\circ$  re the spatial vertical, indicating that it was again closely aligned with gravity.

In summary, these experiments demonstrate that otolith-induced ocular torsion against gravity as well as canal-induced ocular torsion was reduced for sustained periods after reentry. There were no long-term changes in the horizontal VOR after reentry. Although the overall gain of vertical VOR was not changed, there was less up-down asymmetry after landing. Finally, the data support the hypothesis that there is a shift in the yaw axis orientation vector of velocity

storage from a gravitational to a body frame of reference as a result of adaptation to space.

## INTRODUCTION

Adaptation to microgravity presents a unique set of challenges to the nervous system because the input from the canals and the otoliths are no longer in synergy. The demand for head angular movements in spaceflight is essentially unchanged, although stimulation of the semicircular canals with pitch and torsional head movements at high frequencies may be reduced because there is no locomotion (Grossman et al. 1988). Consistent with this, little change has been found in the angular horizontal VOR (see Cohen et al. 1992, for review). There is no information about the VOR in pitch and roll.<sup>1</sup>

On the other hand, the otolith organs face a new set of circumstances. The continuous force of gravity is reduced to a negligible level, although linear accelerations associated with translation persist. (It should be noted, however, that the otoliths do not often encounter comparable force levels in microgravity to those on earth when the head moves in pitch and roll, where it frequently encounters changes in the order of 1g.) Therefore, a reinterpretation of otolith input has been proposed, in which linear force sensed by otolith organs is now interpreted primarily as translation (Young et al. 1986). The consequence is that when the head is tilted laterally (rolled) in microgravity, there should be 1) adaptive down regulation of OCR and 2) no internal representation of the vertical re a gravitational frame of reference.

OCR was measured by an after-image method (Fischer 1927) by Ikovleva and Kornilova. The response of several subjects varied. The reduction in OCR lasted in two subjects for up to 14 days after landing, recovering only at the next test point of 36 days. In some subjects there was anti-compensatory torsion after landing, in the direction of the tilt ("paradoxical counter-rolling", Kornilova; personal communication). OCR was also measured from colored transparencies in the right eye of four subjects after Spacelab-1 Mission (Vogel and Kass 1986). The angles of OCR were expressed as a gain ratio which was reduced for all subjects for left tilt 1 day after landing between 28% and 56%. Whereas, changes in gain on the right were inconclusive.

Rotation about axes tilted from the vertical (off-vertical axis rotation, OVAR) offers unique possibilities as a test for the integrity of canal-ocular and otolith-ocular reflexes. At the onset of rotation about a tilted axis, input to the central nervous system comes both from the semicircular canals and the otolith organs. The canals transduce angular acceleration at the onset and end of rotation, converting it into a signal related to head angular velocity (Goldberg and Fernandez 1971), and the otolith organs sense the linear acceleration (gravity) which continuously changes its direction re head position. From this the nervous system extracts an estimate of head velocity about the tilted axis (Benson and Bodin 1966, Cohen et al. 1983,

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<sup>1</sup>Yaw, pitch and roll head movements are defined in terms of a right hand rule. Yaw movements are those which occur around a head centric axis with movements to the left being positive and movements to the right being negative. Pitch head movements occur around an interaural axis, downward movements being positive and upward movements negative. Torsional movements occur around a naso-occipital axis, counter-clockwise movements from the individual's point of view being positive and clockwise movements being negative. (See Raphan and Sturm, 1991; and Dai et al. 1991; for further description).

Guedry 1965, Raphan et al. 1981, Raphan et al. 1979, Raphan and Schnabolk 1988, Young and Henn 1975). This estimate is utilized to activate the velocity storage integrator to generate continuous nystagmus and compensatory steady state eye velocities (Cohen et al. 1983, Raphan et al. 1981, Raphan and Schnabolk, 1988).

The signal arising in the otolith organs is also used to produce compensatory changes in eye position over otolith-ocular pathways (Baker and Berthorz 1974) as the head changes its position with regard to gravity. At a low angular speed, OVAR can be treated as semi-static stimulation (0.17 Hz at 60°/s, for example). Under this testing condition, as the otolith organs, particularly the utricles, are continuously reoriented with regard to gravity, the magnitude of OCR also changes continuously. The repetitive nature of the dynamic response allows averaging and robust estimation of changes in OCR as a function of gravity, i.e., as the axis of rotation is tilted at various angles from the upright. No other stimulus produces sinusoidal ocular torsion repetitively without involvement of the semicircular canals. Thus, it is possible to gain a dynamic measure of OCR during OVAR as well as a measure of the steady state or bias level of yaw-axis OVAR nystagmus velocity generated through otolith activation.

In addition, there is reason to believe that there could be alterations in spatial orientation after adaptation to microgravity. Dai et al. (1991) and Raphan and Cohen (1988) have demonstrated a mechanism in the vestibular system, "velocity storage", that is spatially oriented on earth. As a result, when yaw axis OKN is elicited with monkeys in tilted positions, the OKAN develops vertical and torsional components that tend to bring the axis of rotation of the eyes toward the spatial vertical. A similar mechanism has been found in humans during OKN (Gizzi et al. 1994). A hypothesis of the current experiments was that adaptation to microgravity would be associated with a shift in orientation of velocity storage from a gravitational axis to a body axis. In the COSMOS 2044 1989 mission, Cohen et al. (1992) found that one flight monkey lost its dumping mechanism after space flight. This supported the notion that the monkey had shifted its internal reference of vertical to its body axis and away from the spatial vertical in the period after reentry.

The current series of experiments was an extension of studies begun on the COSMOS 2044 flight (Cohen et al. 1992). Steps of velocity were used to test the gain of the horizontal and vertical VOR. OCR was elicited by static tilts and by OVAR. Finally, OKAN induced by yaw axis OKN delivered in tilted conditions, was used to test the orientation of the velocity storage system. Preliminary results on OCR have been reported elsewhere (Dai et al. 1993).

## METHODS

### GENERAL:

Eye movements of two rhesus monkeys (6151 and 7906) that flew in space for 12 days on the COSMOS Biosatellite Flight 2229 were compared with their preflight eye movements, with the preflight data from 10 other monkeys that formed a control group, and with data obtained in the postflight period from three of the ten control monkeys. The flight animals were launched on 12/29/92 and recovered on 1/10/93. Testing extended for 11 days postflight.

Eye movements were measured in three dimensions with magnetic scleral search coils. Horizontal and vertical movements were sensed by a coil implanted in the frontal plane by the technique of Judge et al. (1980). Ocular torsion or roll about the optic axis was recorded with a magnetic scleral search coil implanted on the top of one eye using the technique described below. We studied OCR using static tilts of 90° and off-vertical axis rotation (OVAR). Advantages of OVAR are that it affords a measure of dynamic tilt without canal contamination, and that multiple cycles of recording of eye position change can be averaged to obtain a robust measure of alteration in eye position with regard to head position relative to gravity. We also measured the Roll VOR during steps of velocity about a naso-occipital axis with the animal prone and during sinusoidal oscillation with the animal upright.

### SURGERY:

Fourteen male rhesus monkeys of approximately 4 kg were used in these experiments. The animals were also utilized in studies of activity in the vestibular nerve (Manning Correia and colleagues) and studies of linear acceleration on ocular movements (David Tomko and colleagues). Under anesthesia scleral search coils (16mm) were implanted under the conjunctiva in the frontal planes of both eyes to record horizontal and vertical eye position, and a roll coil was implanted on the top of the left eye of each animal to record torsional eye movements.

To implant the roll coil, the left eye was pulled down, and a lateral cut was made in Tenon's capsule about 2 mm below the tarsal plate. The dissection was carried down to the superior rectus muscle, which was identified and freed both medially and laterally. A three turn 12 mm coil was fashioned by passing the wire under the superior rectus muscle. The coil was pushed into place posteriorly, where it lay under the superior rectus muscle on the top of the eye. Anteriorly, it was sutured in place at about the level of the equator. A tension loop was pushed up into the pocket lateral to the superior rectus, and the coil wires were led through the lateral portion of Tenon's capsule.

The conjunctiva was then dissected free about 1 mm from the edge of the limbus, a deep pocket was opened with blunt and sharp dissection, and a 16 mm 3 turn coil was placed over the eye and sutured in place. A tension loop was placed into the pocket, lateral to the inferior rectus muscle, and the wires were led through the lateral portion of Tenon's capsule. The same frontal plane coil operation was carried out on the right eye without difficulty or special bleeding. The coil wires were led out of the orbit and sutured to the fascia overlying the temporal muscle, before being led to the top of the skull, where they were attached to a plug. Typical impedances of the coils were: Left eye roll coil 56 ohms; frontal eye coil 67 ohm.

## EXPERIMENTAL APPARATUS:

A four axis vestibular and optokinetic stimulator (Neurokinetics of Pittsburgh) was transported from New York to the Institute of Biomedical Problems in Moscow for these experiments. The apparatus provided great flexibility to accomplish a variety of experimental paradigms. It had four axes driven by servo-motors that were independent and were under computer control. A picture of the apparatus is shown in Fig. 1. The animal rotator was enclosed in an optokinetic sphere, 122 cms in diameter, with  $10^\circ$  vertical black and white stripes. When rotated, the sphere produced full field motion that induced OKN. The rotator consisted of a "C" gimbal, through the center of which was a rotational axis to which the primate chair was attached. We shall designate the latter as the "chair axis". The chair axis was positionally controlled to provide  $\pm 90^\circ$  of excursion. It produced pitch or roll of the animal, depending on whether the axis of rotation was coincident with the animal's interaural or naso-occipital axis, respectively. The gimbal axis produced rotation of the animal about an axis coincident with the axis of the surrounding optokinetic sphere. The gimbal axis could be controlled in either velocity or positional servo mode. The maximum acceleration and deceleration of the C gimbal and optokinetic sphere were  $200^\circ/\text{s}^2$ . The sphere and C gimbal were fixed to a spine that surrounded the sphere and was attached to two lateral posts. The spine was positionally controlled to pivot about a horizontal axis  $\pm 180^\circ$ .

The animal's chair could be positioned in four directions,  $90^\circ$  apart, with regard to the chair axis. For example, if it was facing out or in along the axis of rotation, the animal could be rotated around by  $\pm 90^\circ$  about its naso-occipital axis. Likewise, if it were left or right ear in, it could be pitched by  $\pm 90^\circ$  about its interaural axis. The monkey could also be continuously rotated with velocity steps around the primate axis. If it was upright, the rotation axis would be along the animal's head-feet (Z) axis. If the animal was tilted  $90^\circ$  on its side, rotation was around its interaural axis. If it was tilted  $90^\circ$  into a prone or supine position, the rotation would be along a naso-occipital axis. All these rotations were head-centered.

Although the C gimbal and optokinetic sphere axes were coaxial, they were independent of each other. This allowed us to rotate the visual surround and monkey at the same rate in some experiments to produce a relative stationary surround. In this paradigm, the vestibular system indicates that the head is moving, but the visual system indicates that it is stationary. We designate this as a "relative stationary surround". As a result, vestibular nystagmus is quickly suppressed, particularly that component attributable to velocity storage (Raphan et al. 1979; Waespe et al. 1983). The ability to rapidly suppress or "dump" nystagmus proved valuable in shortening the time of experiments during the postflight testing.

A final modification of the chair enabled us to position the animal about 38 cms from the axis of rotation. This provided the capability of centrifugation. Preliminary experiments were carried out using centrifugation at  $200^\circ/\text{s}$  in the head forward and backward conditions and with the animal facing outward. Under this velocity, a resultant force of about  $18^\circ$  re gravity was produced. Adequate ground-based data is not available for comparison with these results, and they will not be included in this report.

Thus, the entire apparatus could be tilted to any position while the animal pitched or rolled, or while the optokinetic sphere provided visual stimulation or the animal rotated about any axis through the center of the head.



## EXPERIMENTAL CONTROL AND DATA ACQUISITION:

Eye position was recorded by a magnetic scleral search coil technique (Robinson 1963). During experiments animals sat in a primate chair. Their heads were fixed to a square plastic frame, 33 cms on a side, that held the field coils and was attached to the primate chair. The eyes were centered in the field coils. Voltages associated with eye position and with the position and velocity of the various axes were recorded through analog filters with a bandwidth of DC to 40 Hz. Eye positions, analog-differentiated velocities and stimulus data were displayed on a 8 channel chart recorder (Astromed) and an oscilloscope. A 486 PC computer was used to control the experiment and to take the data. Each stimulus paradigm was pre-designed and called in by a computer program specially designed for that experiment. The voltages associated with eye positions were sampled at 600 Hz and stimulus data were sampled at 150 Hz.

All settings of analog instruments remained the same throughout the Mission. The resistances of eye coils were measured in all experiments and there was no change before and after space flight. Therefore, we were able to compare eye movements of animals before and after flight.

## EXPERIMENTAL PARADIGM:

Prior to all tests, yaw, pitch and roll velocity calibrations were done. The monkeys were placed upright, left ear 90° down and in prone positions, respectively, and rotated about a vertical axis at  $\pm 30^\circ/\text{s}$  for 20 seconds while they viewed the stationary OKN stripes in light. Under this condition, the velocity gain (slow phase eye velocity/head velocity) of the combined visual-vestibulo-ocular reflex was assumed to be close to unity for the yaw and pitch eye velocities. The roll velocity gain was determined by comparing the output of the implanted roll coil from each monkey during torsional nystagmus to that of a similar "dummy" coil of the same size, mounted on a jig and moved in the same magnetic field. A positional calibration was first established for the "dummy" coil. This was then used to calibrate torsional eye position changes during the nystagmus, and to compare the velocity of the slow phases to the velocity of the stimulus. Using this technique the gain of the torsional VOR was about 0.6 for all monkeys. This agrees with data from other laboratories on the gain of the roll VOR in rhesus monkeys (Henn, personal communication). Regardless, it should be emphasized that the coil amplifier settings were the same before and after flight, and the coil resistances were unchanged. Therefore, the pre and postflight results could be compared to each other.

OCR was tested in two ways after a calibration in which the animal was rotated around a naso-occipital axis coincident with gravity while prone and in light. To test static OCR, the monkey was positioned upright in the darkness for 20 seconds and then tilted 90° to its left and right for another 20 seconds, respectively. Spontaneous torsional eye position were recorded for each of the three conditions. OCR was also studied during OVAR nystagmus induced by rotation at 60°/s. The experiment was done in the following way: First, the animal was upright and was rotated in light with a relative stationary surround, i.e., with the optokinetic sphere moving at 60°/s to dump the nystagmus induced by the semicircular canals at the onset of rotation. After the nystagmus had disappeared, the lights were turned off while the animal continued to rotate. The rotating axis was subsequently tilted with a speed of less than 5°/s to angles of 15°, 30°, 45°, 60°, 75°, 90°, 105°, 120°, 135°, 150°, 165°, 180°, 195°, 210°, 225°, 240°, 255°, 270°, 285°, 300°, 315°, 330°, 345°, and 360°.

45°, 60°, 75° and 90° re gravity. Each tilt of the rotation axis was held for 36 seconds (6 cycles of rotation). During OVAR compensatory horizontal nystagmus developed and reached a steady state level. Since the animal's head was reoriented continuously with respect to gravity during OVAR, roll eye position was altered continuously in direction and magnitude (ocular counter rolling) at a frequency of 0.17 Hz. Two series of tests were done utilizing OVAR, one with animal rotating to its right and one to its left.

Animals were tested in two different experimental paradigms to evaluate the gain of the roll VOR. In the first paradigm, the animal was placed prone and rotated with a velocity step ( $200^\circ/\text{s}^2$ ) about its naso-occipital axis in the darkness, at angular speeds of  $\pm 30^\circ/\text{s}$ ,  $\pm 45^\circ/\text{s}$ ,  $\pm 60^\circ/\text{s}$ ,  $\pm 75^\circ/\text{s}$  and  $\pm 90^\circ/\text{s}$ . In the second paradigm, the animal was upright and was oscillated sinusoidally over  $\pm 30^\circ$  around its naso-occipital axis which was horizontal at frequencies of 0.025, 0.05, 0.077, 0.1 and 0.125 Hz. The cycles of sinusoidal oscillation varied, ranging from 5-10 cycles per frequency.

The horizontal and vertical VOR were tested in the conventional way. For the horizontal VOR, the monkey was in an upright position, and it was rotated at angular speeds of  $30^\circ/\text{s}$ ,  $60^\circ/\text{s}$ ,  $90^\circ/\text{s}$  and  $120^\circ/\text{s}$  about a spatial vertical axis which was coaxial with body axis. For the vertical VOR, the monkey was positioned left side  $90^\circ$  down and rotated with angular speeds of  $30^\circ/\text{s}$ ,  $45^\circ/\text{s}$ ,  $60^\circ/\text{s}$ ,  $75^\circ/\text{s}$  and  $90^\circ/\text{s}$  around its interaural axis, which was spatially vertical. The VOR gain is flat at these velocities (Raphan et al. 1979; Matsuo and Cohen 1984).

#### DATA ANALYSIS:

A velocity calibration was used to obtain a relative calibration for horizontal and vertical eye position. The individual slope of slow phases of eye position was calculated over the period of velocity calibration, and the slopes of eye position were averaged. The mean was then converted to a  $15^\circ$  eye position calibration. The baseline or zero eye position was determined by averaging eye position over a long period of spontaneous eye movements with the animal upright. For the calibration of horizontal and vertical eye movement, the gain, defined as eye velocity divided by stimulus velocity, was taken as unity (Cohen 1992). As noted above, the gain for roll eye velocity was about 0.6. The preflight calibration was used throughout, since the gain of the postflight response might differ from the preflight response. Data analyses for both eye position and eye velocity were based on the converted eye position calibration.

When calculating static OCR, torsional eye positions were considered only if the eyes were not deviated more than  $\pm 10^\circ$  horizontally or vertically from the midposition. The values of OCR for the monkey tilted  $\pm 90^\circ$  laterally were referenced to the baseline torsional position in the upright position.

Dynamic OCR was also recorded in tilt positions from  $15$ - $90^\circ$  during OVAR. 5 to 6 cycles of roll eye position data were averaged during OVAR nystagmus. The trigger signal came from the output of a potentiometer which registered the yaw axis rotation position. The mean roll eye position then was fitted with a sine function. The peak value of the fitted sine curve was taken as the amplitude of the OCR.

In the data analysis associated with slow phases of eye velocity, eye position data were digitally differentiated, and quick phases of nystagmus were removed by a histogram desaccading algorithm. The data was then smoothed by averaging each four points, which would reduce the

sampling rate to 150 Hz. To calculate the gain of the horizontal, vertical or roll VOR from velocity steps about a vertical axis, the slow phases associated with first three nystagmus were marked and averaged, and then the gain was obtained by dividing mean eye velocity by the corresponding roll step stimulus velocity.

To calculate the gain of roll eye velocity from the naso-occipital oscillation about a horizontal axis, the displayed eye velocities were first desaccaded. 5-10 cycles of slow phases of eye velocity were averaged, and the averaged data were fitted by a sinusoidal function. The peak value of the fitted curve was taken as the peak amplitude of the roll eye velocity. The gain then was obtained as the ratio of peak roll eye velocity versus peak oscillating velocity of the stimulus.

## RESULTS

### STATIC AND DYNAMIC OCULAR COUNTER-ROLLING (OCR):

Static OCR in response to tilts of  $\pm 90^\circ$  are shown in Table 1. Although there was no difference in the magnitude of OCR between left and right tilts across the five monkeys preflight and for the three controls postflight (right  $6.4^\circ \pm 0.9^\circ$ , left  $5.7^\circ \pm 1.0^\circ$ ;  $p=0.069$ ), we considered each side to be a separate trial in the statistical calculations. A striking finding was that OCR was dramatically reduced by 70% after recovery from spaceflight in both flight monkeys, and there was no apparent recovery in the magnitude of torsion over 11 days of testing ( $p < 0.001$ ,  $n1=10$ ,  $n2=10$ ). In contrast, there was no change in the ocular torsion of the three control monkeys when compared in the preflight and postflight period ( $p=0.92$ ,  $n1=10$ ,  $n2=6$ ).

Dynamic OCR was assessed using OVAR. The roll component of eye movement showed a typical increase in the amplitude of sinusoidal modulation before flight as a function of tilt of the angle of rotation (see Fig 1A, ROLL POSI). In contrast, the magnitude of OCR was substantially reduced after flight (Fig. 2B, ROLL POSI). Changes shown in Fig. 2B for 6151 were similar to those found in 7906 and can be contrasted to the preflight OCR and the findings in the control monkeys. In each of the animals there was approximately  $\pm 7^\circ$  of torsion when the axis of rotation was tilted  $60^\circ$ - $90^\circ$  in the preflight or control postflight testing (Fig. 3A-E), whereas the maximum torsion induced by OVAR after flight was  $2^\circ$ - $3^\circ$  (Fig. 3D, E).

The reduction of dynamic OCR in the entire group is summarized in Fig. 3F. The preflight OCR for the two flight monkeys fell more than two standard deviations below the preflight mean of 5 monkeys, a highly significant difference ( $p < 0.001$ ,  $n1=5$ ,  $n2=7$ ). The reduction of dynamic OCR for 7906 was more than for 6151. Nevertheless, there was no recovery of OCR for both monkeys over the post-test period of 11 days. As with static OCR, the reduction in dynamic OCR after flight was 70%.

We considered various artifacts that might account for the reduction in OCR in the flight monkeys. Changes in the apparatus were ruled out by the same values for OCR pre and postflight for the control monkeys (Fig. 3A-C). It was postulated that there might be adhesions in the supraorbital area around the roll coil in the flight monkeys that was tethering the eye. One of the animals was anesthetized, and its eyes were tortured physically by grasping the conjunctiva at both sides of the limbus and moving the eye in roll. There was no difference in the physical force needed to move either eye. Moreover, adhesions that might have reduced the amount of eye movement before the mobility test and been broken by the physical movement were ruled out because there was no difference between the OCR before and after the mobility test. This demonstrated that the eye was not tethered in roll for these two monkeys after flight.

### ROLL VOR:

The Roll VOR was measured in two experimental paradigms, with velocity steps with the monkeys prone while rotating about a vertical naso-occipital axis (Fig. 4A) and with sinusoidal oscillations with the monkeys upright rotating about a horizontal axis (Fig. 4B). The gain of the Roll VOR of 7906 was reduced about 50% and of 6151 about 15% in both modes

of stimulation. Especially for 6151, this was less than the amount of reduction in OCR, which was about 60%. However, with the exception of 6151 at 30°/s step and 0.025 Hz oscillation, it was significantly different from the preflight means across all step velocities and frequencies, laying more than  $\pm 2SD$  from the means of the five monkeys. In comparison, there was no reduction in the gain of the Roll VOR in the three control monkeys.

There were some differences between the two tests. In the velocity step test (Fig. 4A), only the vertical semicircular canals were stimulated, and there was no contribution from the otoliths, but activation was at a high frequency. In the oscillation experiments both the vertical canals and otoliths were activated to generate roll movements, but the mode was with low frequencies (0.025-0.125 Hz). This may account for the finding that the overall response to oscillation had a lower gain than that to the velocity step.

#### VERTICAL VOR:

There was upward spontaneous nystagmus in both monkeys before flight. This is a common finding in normal rhesus monkeys. The spontaneous nystagmus in 6151 can be seen in the vertical velocity trace of Fig. 2, A. The spontaneous nystagmus was reduced when the animal was first tested, 2 days after reentry (Fig. 2, B). This was also true for 7906 when tested 1 day after landing.

The upward VOR gain of the monkeys before flight was  $0.96 \pm 0.03$ . The downward gain was  $0.75 \pm .04$ . Thus both monkeys had an asymmetry of their VOR gains before flight, with the upward gain being greater than the downward gain. This was the same as other monkeys in the control pool. In the first postflight record, there was a decrease in the gain of the upward VOR to  $0.90 \pm 0.03$ , and an increase in the downward VOR to  $0.82 \pm 0.03$  for 1-2 days for both monkeys<sup>2</sup>. These differences were small but statistically significant (decrease in upward gain: 7906,  $p=0.04$ ,  $n=5$  and 6151,  $p=0.01$ ,  $n=5$ ; increase in downward gain: 7906,  $p=0.04$ ,  $n=5$ ; 6151,  $p=0.02$ ,  $n=5$ ). If the gains for the up- and downward VOR were considered together, the differences in pre- and postflight gains equalized, and there was no difference in either 7906 ( $p=0.78$ ,  $n=10$ , day 1) or 6151 ( $p=0.74$ ,  $n=10$ , day 2) before and after flight.

By 7 days of landing, there was no statistical difference in gain between pre- and postflight testing for the upward VOR in both monkeys (7906:  $p=0.07$ ,  $n=5$ ; 6151:  $p=0.12$ ,  $n=5$ ) or for the downward VOR in 6151 ( $p=0.81$ ,  $n=5$ ). Monkey 7906 still had an increased downward VOR gain ( $p=0.03$ ,  $n=5$ ).

In summary, there was a drop in the upward VOR gain 1-2 days after landing and an increase in the downward VOR gain. By 7 days the gain had returned to preflight level except for an increase in the downward VOR gain in one animal.

#### HORIZONTAL VOR:

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<sup>2</sup>Monkey 7906 was tested on Day 1 postflight, 22 hours after landing. Monkey 6151 was tested on Day 2 postflight, 55 hours after landing.

The gain for the horizontal VOR was pooled for rotation to the right and left, since there was no gain difference between them. As shown in Table 3, there was no yaw gain difference between pre- and postflight testing, 1-2 days after landing for both 7906 ( $p=0.65$ ) and 6151 ( $p=0.22$ ). This confirms findings reported in the 1989 Mission (Cohen et al 1992). There was also no change in the gain of the steady state horizontal velocities during OVAR, which were the same before flight and after landing (Fig. 2A, B, Horizontal Velocity).

#### CHANGES IN SPATIAL ORIENTATION:

The yaw axis eigenvector of OKAN, derived from the feedback matrix of a velocity storage integrator, has been regarded as an internal representation of the vertical (Dai et al. 1991; Raphan and Sturm 1991). Before flight, the eigenvector angle re gravity for 7906, tested during yaw axis OKN at 90° of tilt, was 5° (Fig. 5A). This was close to the spatial vertical and was similar to other normal monkeys tested in a previous study (Dai et al 1991). Twenty-two hours after landing, the yaw axis eigenvector had moved significantly away from spatial vertical and was now 28° (Fig. 5B). This is consistent with the hypothesis that there is a tendency for the spatial orientation of OKAN to move toward a body axis as a result of adaptation to microgravity. By 7 days after landing, the eigenvector angle was 7°, having returned to its preflight level (Fig. 5C). Unfortunately, testing of the orientation of velocity storage was limited in 6151 after flight because it was sick after recovery and could not be tested until the second day, at which time there was no alteration in the spatial orientation.

## DISCUSSION

The major finding of these experiments is that the torsional otolith-ocular reflex, induced by head tilt with regard to gravity, was substantially reduced in two flight monkeys after adaptation to space, and that the reduction in OCR persisted for a prolonged period after reentry. There was also a reduction in the gain of the Roll VOR, more in one monkey than in the other. The alteration in Roll VOR gain was to a lesser extent in both monkeys than the changes in OCR. The reduction in the gain of OCR measured dynamically during OVAR was the same as the reduction in gain of static OCR, measured in side down position. Thus, regardless of how the monkey was moved to a side down position, the counter-rolling in that position was reduced after spaceflight. In addition, the reduction in gain was not dependent on the yaw orientation vector of velocity storage but remained uniformly lower despite the fact that the orientation vector changed from  $5^\circ$  to  $28^\circ$  back to  $7^\circ$ . This supports the notion that the gain of the direct otolith ocular pathway is separate from the pathway responsible for velocity storage.

There are two types of afferents from the otoliths. One is phasic, carrying irregular activity from large fibers that innervate Type I hair cells that lie close to the striola. The firing rates of these cells typically adapt when the head is statically tilted, making them poor sensors of head position. However, their activity could be integrated to generate the position command for driving motoneurons (Cannon and Robison 1987; Scknabolk and Raphan 1993). They also have a steep gain curve. As a result, their pulse-like activity could summate with the output of the integrator to be useful during translation. The second type of afferent is generally of smaller diameter and carries regular fibers from Type II hair cells. These cells do not adapt, making them excellent sensors of head position. The gain adaptation in OCR that occurred in the two monkeys may have been the result of a reduction in the effect of the regular afferents, acting on direct otolith-ocular pathways. In addition, the roll gain control of the final position integrator (Cannon and Robinson 1987) as well as the cerebellum (Zee et al. 1980) may also participate in producing the adaptation in space.

The finding that the steady state levels of OVAR were not affected by spaceflight indicates that the otolith organs were sensing gravity correctly, and that they were putting input into circuits that activated velocity storage to produce horizontal eye movement and yaw axis eye velocity without difficulty. This implies that the functional structure of the otolith and its phasic afferents were not changed after flight. On the other hand, there were indications that canal-ocular as well as otolith ocular torsional reflexes were adapted. This is surprising, since in these same animals, there was no change in the horizontal VOR gains, tested in a similar fashion. The adaptation of OCR was long-lasting, although the alteration in the spatial orientation of velocity storage recovered more quickly. This indicates that they are dependent on different mechanisms.

What is the reason for the striking changes in OCR as well as the significant, although less striking changes in the Roll VOR? Torsional head movements occur at low frequencies on earth, but probably the largest stimulus to torsional movement is lateral head tilt which activates OCR through gravity. In space, horizontal and vertical eye movements can be generated voluntarily or through canal reflexes. On the other hand, there are no voluntary torsional eye movements, and only negligible gravity is present. Therefore, maintenance of the roll reflex would depend only on roll-angular accelerations. One possibility is that there was relative

inactivity of the torsional system in space, leading to a reduction in gain after reentry. Since the monkeys used in the COSMOS project were chaired and were not able to exercise freely, they may have made even fewer torsional head movements than astronauts or cosmonauts. If the changes in OCR were related to inactivity, there might be differences in the amount of OCR between monkeys and humans who were able to move more freely. Were this to be substantiated in future experiments, a program of head movements which produced compensatory torsional ocular movements might be a natural counter-measure.

While we hypothesize that the adaptation of otolithic pathway in microgravity environment affects the roll eye movement as well as the internal reference of the spatial vertical, another finding of the change in vertical VOR gain may also be the result of otolithic pathway adaptation. In this study, we have shown that the upward VOR gain was reduced and the downward VOR gain was enhanced while the upward spontaneous nystagmus was decreased after landing. It is possible that the spontaneous level of vertical nystagmus reflects the asymmetry of vertical VOR gain.

The otolith organs may also play a role in spontaneous vertical eye movement. The present data show that the level of spontaneous vertical nystagmus varied after spaceflight, and the asymmetry in vertical slow phase velocity was reduced. Clement and Berthoz (1990) demonstrated that there was a downward drift of the eyes after adaptation to spaceflight in humans. The two may be related. Unfortunately, the effects of otolith on vertical eye movement have not been thoroughly studied.

There are several steps that would aid in understanding these results: The reduction in OCR should be replicated in monkeys and studied in humans, and it should be correlated to behavior after landing. Motion analysis of head movements should be done to provide information about the content of natural head movements in space and after landing, particularly those around a naso-occipital axis. We would predict that there would be fewer torsional head movements in space than on earth. If a similar decrease occurs in OCR in humans as in monkeys, tasks should be designed so that they do not involve the necessity for torsional eye velocities or changes in torsional eye position. Counter-measures, such as a program of torsional head movements in space, might be considered. Finally, the gain of the vertical and roll VOR should be studied systematically, as well as the equalization of the vertical asymmetry.



## BIBLIOGRAPHY:

- Baker, R., Berthoz A. (1974) Organization of vestibular nystagmus in oblique oculomotor system. *J. Neurophysiol.* 37: 195-217.
- Cannon, S.C. Robinson, D.A (1987) Loss of the neural integrator of the oculomotor system from brain stem lesions in monkey. *J Neurophysiol* 5: 1383-1409.
- Clement G, Berthoz A (1990) Cross-coupling between horizontal and vertical eye movements during optokinetic nystagmus and optokinetic afternystagmus elicited in microgravity. *Acta Otolaryngol.(Stockh)* 109:179-187
- Cohen B., Suzuki J. I., Raphan T. (1983). Role of the otolith organs in generation of horizontal nystagmus: effects of selective labyrinthine lesions. *Brain Res*, 276: 159-164.
- Cohen B., Kozlovskaya I., Raphan T., Solomon, D., Helwig, D., Cohen, N., Sirota, M., Yakushin, S. (1992). Vestibular reflex of rhesus monkeys after spaceflight. *J App Physiol (Supplement)*, 73: 121-131.
- Dai M., Raphan T., Cohen B. (1991). Spatial orientation of the vestibular system: Dependence of optokinetic after-nystagmus on gravity (Abs). *J. Neurophysiol.* 66: 1422-1439.
- Dai M.J., Raphan, T., Coen B (1991) Spatial orientation of velocity storage during post\_rotatory nystagmus (Abs). 21st Neuroscience Annual Meeting, Part 1, pp 314.
- Dai, M.J., Cohe, B., Raphan, T., McGavie, L., Kozlovskaya, I.B (1993) Reduction of ocular counter-rolling (OCR) after spaceflight (Abs). 23rd Neuroscience Annual Meeting, Part1, pp 343.
- Benson A.J., Bodin M.A. (1966) Interaction of linear and angular accelerations on vestibular receptors in man. *Aerosp Med* 37: 144-154.
- Fischer M.H (1927) Messende Untersuchungen uder die Gegenrollung der Augen und die Lokalisation der scheinbaren Vertikalen bei seitlicher Neigung des Gesamtkorpers bis zu 360°. I. Mitteilung. Neigung biz zu 40° (Untersuchungen an Normalen). *Albrecht von Graefes Arch Klin Ophthalmol.* 118, 633.
- Gizzi M., Raphan T., Cohen B (1993) Orientation of human optokinetic nystagmus (OKN) to gravity: A model based approach (Abs). 23rd Neuroscience Annual Meeting, Part1, pp 343.
- Goldberg J. M., Fernandez C. (1971). Physiology of the peripheral neurons innervating semicircular canal of the squirrel monkey. I. Resting discharge and response to constant angular accelerations. *J Neurophysiol* 34: 635-660.

- Grossman G. E., Leigh R. J., Abel L. A., Lanska D. J., Thurston S. E. (1988) Frequency and velocity of rotational head perturbations during locomotion. *Exp. Brain Res.* 70: 470-476.
- Guedry F.E. (1965) Orientation of the rotation axis relative to gravity: its influence on nystagmus and the sense of rotation. *Acta Otolaryngol (Stockh)* 60: 30-48.
- Judge S.J., Richmond B.J., Chu F.C. (1980) Implantation of magnetic search coils for measurement of eye position: an improved method. *Vision Res* 20: 535-538.
- Matsuo V., Cohen B., Raphan, T., DeJong V., Henn V (1979) Asymmetric velocity storage for up and down nystagmus. *Brain Res* 176: 159-164.
- Matsuo V and Cohen B (1984) Vertical optokinetic nystagmus and vestibular nystagmus in the monkeys: Up-down asymmetry and effects of gravity. *Exp Brain Res* 53: 197-216.
- Raphan T., Matsuo V., Cohen B. (1979) Velocity storage in the vestibulo-ocular reflex arc (VOR). *Exp Brain Res*, 35: 229-248.
- Raphan T., Cohen B., Henn V. (1981) Effects of gravity on rotatory nystagmus in monkeys. *Annals of the New York Academy of Sciences*, 374: 44-55.
- Raphan T and Cohen B (1988) Organizational principles of velocity storage in three dimensions: The effect of gravity on cross-coupling of optokinetic after-nystagmus. *Annals of the New York Academy of Sciences*, 545: 74-92.
- Raphan T., Schnabolk C. (1988) Modeling slow phase generation during off-vertical axis rotation. *Ann NY Acad Sci* 545: 29-50.
- Raphan T and Sturm D (1991) Modeling the spatiotemporal organization of velocity storage in the vestibuloocular reflex by optokinetic studies. *J Neurophysiol.* 66: 1410-1421.
- Robinson D. A. (1963). A method of measuring eye movement using a scleral search coil in a magnetic field. *IEEE Trans Biomed Eng* 10: 137-145.
- Schnabolk, C and Raphan T (1993) Model of three dimensional velocity-position transformation in oculomotor control. 23rd Neuroscience Annual Meeting, Part2, pp 1593.
- Waespe W., Cohen B., Raphan T. (1983) Role of the flocculus and paraflocculus in optokinetic nystagmus and visual-vestibular interactions: effects of lesions. *Exp Brain Res* 50: 9-33.
- Vogel H and Kass J.R (1986) European vestibular experiments on the Spacelab-1 mission: 7. Ocular counterrolling measurements pre-and post-flight. *Exp Brain Res* 64: 284-290.

Young L.R and Henn V. (1975) Nystagmus produced by pitch and yaw rotation of monkeys about non-vertical axes. Fortschr Zool 23: 235-246

Young L.R., Oman C.M., Watt D.G.D., Monney K.E., Lichtenberg B.K., Kenyon R.V (1986) M.I.T./Canadian vestibular experiments on the Spacelab-1 mission: 1. Sensory adaptation to weightlessness and readaptation to one-g: an overview. Exp Brain Res 64: 291-298.

Zee D.S., Leigh R.J., Mathieu-Millaire F (1908) Cerebellar control of ocular gaze stability. Ann Neurology 7:37-40.

## LEGENDS:

FIG. 1, Four axis vestibular and optokinetic stimulator used in this series of experiments. See Methods for complete description of the apparatus.

FIG. 2, Nystagmus induced by rotation in darkness at  $60^\circ/\text{s}$  about a tilted axis darkness in M6151 before (A) and 7 days after (B) spaceflight. The animal was first rotated at  $60^\circ/\text{s}$  about a vertical axis with the OKN drum in light inducing per-rotatory nystagmus whose slow phase velocity declined steadily to zero (not shown). Then, the axis of rotation was tilted from  $15$ - $90^\circ$  (Tilt Pos, first trace), while the yaw axis rotation continued (Yaw Pos, second trace), inducing nystagmus. Roll, vertical and horizontal eye velocity and position are shown, from top to bottom. Note the modulations in slow phase velocity (H Vel, fifth trace) and in roll eye position (Roll Posi, fifth trace) during the OVAR. Note the decrease in the modulation of roll position after flight (B). Note also that the steady state horizontal velocity was achieved both before and after flight, and that there was a larger modulation in horizontal velocity as a function of gravity after flight.

FIG. 3, A-E, Graphs of preflight and postflight dynamic OCR induced by OVAR in three control monkeys (A-C) and the two flight monkeys (D-E). Five cycles were averaged for rotation to the right and to the left, and the means were combined into the value shown in the graph. The vertical error bar is one standard deviation. Each control animal had a test 60-90 days before flight and after flight. The flight animals had a control test 60-90 days before flight and several tests after flight from the first (D1) to the eleventh day (D11). F, Mean and 2 standard deviation (shaded area) for control data from the 3 control animals and the preflight values for the flight animals. Note that the postflight data fell more than two SD from the mean at all tilt angles. This was a highly significant difference in unpaired t-tests.

FIG. 4A, Graph of gain of Roll VOR induced by steps of velocity between  $30$  and  $90^\circ/\text{sec}$ . The mean for the preflight testing is shown by the heavy solid line (Pre-mean), which is surrounded by a shaded area representing  $\pm 2\text{SD}$ . There was a slight decrease in Roll VOR gain in M6151 after flight, and a larger decrease in the Roll VOR of M7906. B, Graph of gain of Roll VOR induced by sinusoids around a horizontal interaural axis. The shaded area shows the preflight mean  $\pm 2\text{SD}$ . After flight there was a decrease in the gain of the Roll VOR, more for 7906 than for 6151.

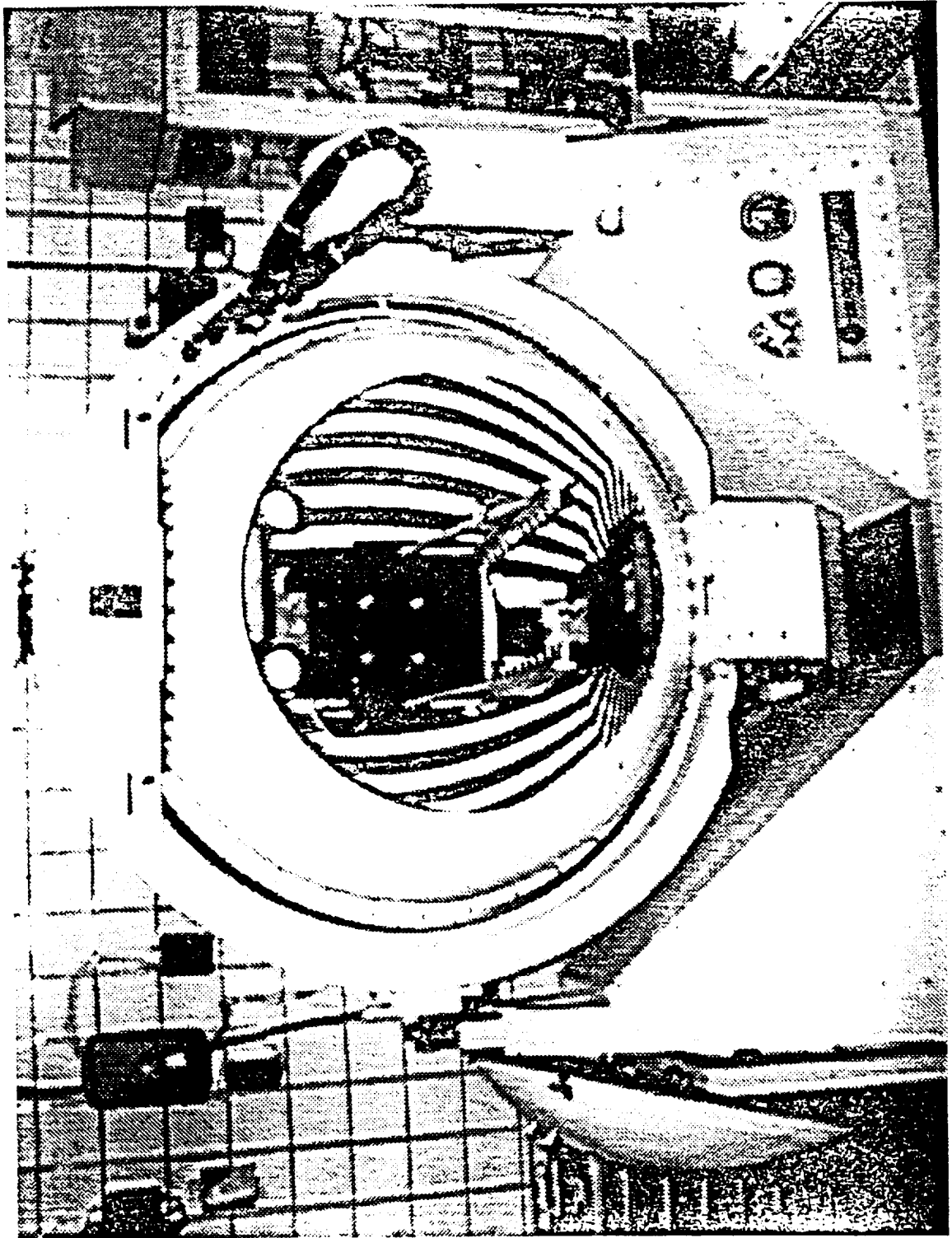
FIG. 5, Phase plane graphs of vertical (ordinate) versus horizontal (abscissa) eye velocity for OKAN induced by yaw axis rotation with M7906 in a  $90^\circ$  tilted side down position. OKAN slow phase velocities began in each graph on the right and the velocities progressed toward zero in a curved fashion. The circles each represent the velocity of one slow phase. The solid curved line represents the fit of the data using a modified Levenberg, Marquardt algorithm. The straight line is the trajectory of the last part of the graph and shows the slope of the fitted curve as the data approached zero. In A, before flight, the yaw axis eigenvector had an angle of  $5^\circ$  from the vertical. B, Twenty-two hours after flight, the slope of the yaw axis eigenvector had shifted toward the body axis and was now  $28^\circ$  from the vertical. C, Several days later, the yaw axis

eigenvector had returned to close to its original angle and now was deviated  $7^\circ$  from the vertical.

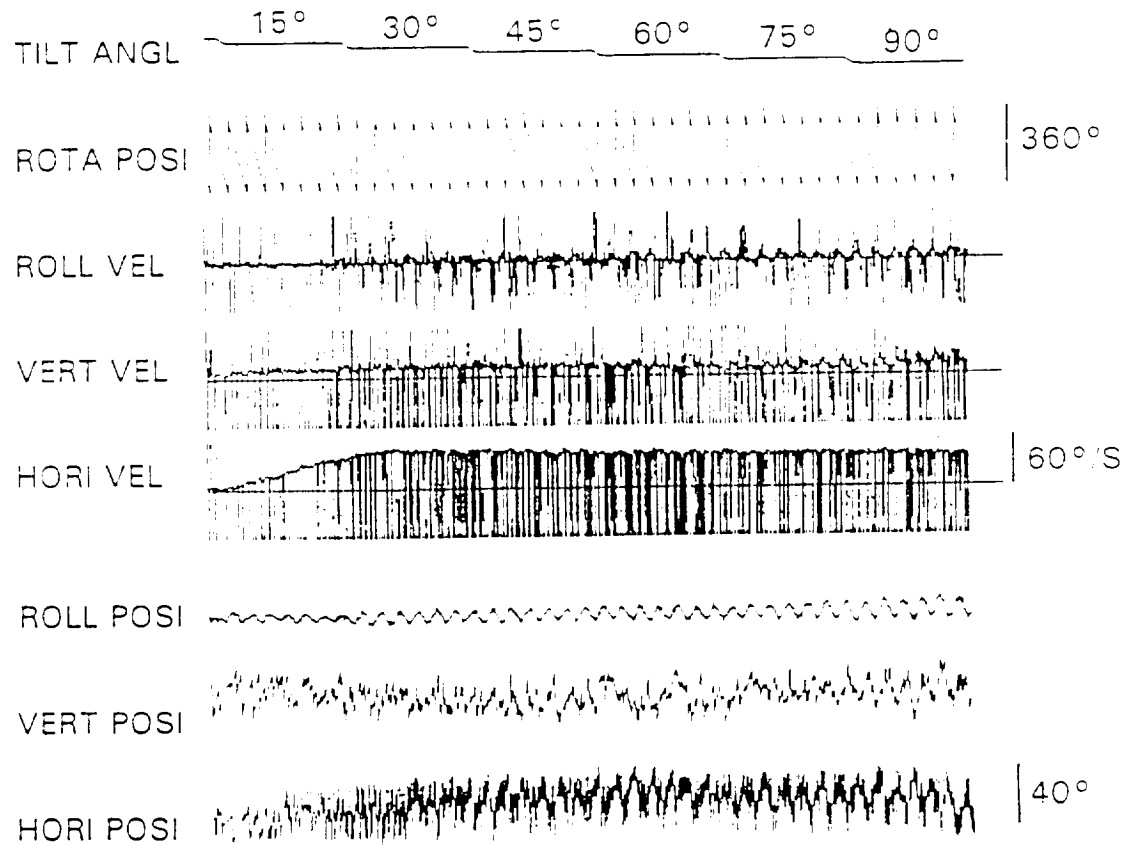
TABLE 1: Static OCR in two flight monkeys (A) and three control monkeys (B). Shown are the average deviation of the eyes before flight for 7906 and 6151 (Pre) and in the preflight period for the three control animals, 7803, 7907, and 5775 (Pre). The Post value for the control animals in B represents OCR taken in the postflight period. It was not different than that in the preflight period. In contrast, there was a substantial reduction in OCR (70%) postflight.

TABLE 2: Gains of the vertical VOR in the per- and postflight periods. The gains for the up- and downward VOR were listed separately due to the asymmetry of the vertical VOR gain before flight.

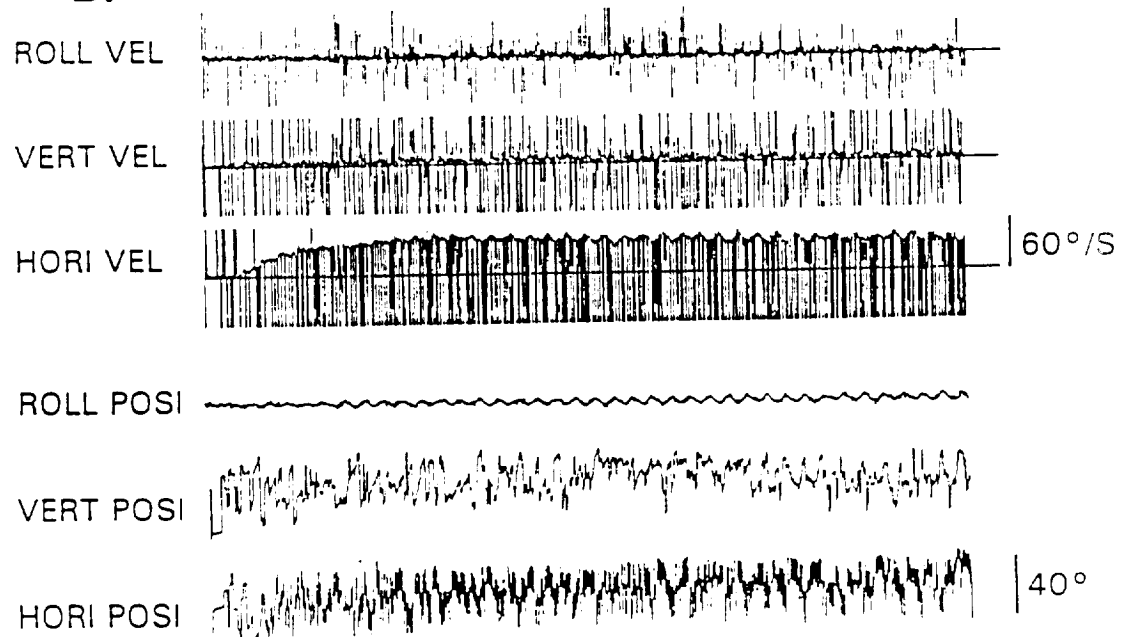
TABLE 3: Gains of the horizontal VOR in the per- and postflight periods for monkeys. The gain for the horizontal VOR was averaged for the rotations to the right and to the left, since there was no gain difference between the two rotations.



A.



B.



30 SEC

